Structural changes in attaching sites of tendon of femoral quadriceps muscle in growing rats

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Summary

Ends of tendon fibbers are embedded in a bone from an embryonic period, and an extent and a depth of the embedding portion change with the growth. However, a relationship between their structural changes with growth and a pathogenesis of the growth disease hadn't been reported. This study aimed to investigate morphologically the structural change in a tibial tuberosity with growth using rats.

Twenty- four male rats (3, 7 and 13-week-old) were used as materials. Their tibias were excised, various specimens were made and they were observed.

An anterior end of an epiphyseal cartilage that was going to be the tibial tuberosity in future already protruded at the stage of 3-week-old. Many fiber bundles of patella tendon attached on the surface of the epiphyseal cartilage and were embedded in the cartilage as Sherpey's fibers. The extent and the depth of the portion that the Sharpey's fibers were embedded increased with growth. A calcification of the cartilage of the tibial tuberosity progressed gradually from deep area, but the surface of that was composed of the cartilage at the stage of 3-week-old yet.

It was suggested that the calcification of the protruding portion of the epiphyseal cartilage that was going to formed the tibial tuberosity in the future was delayed compared to the other portions.

Keywords: Patella tendon, Tibial tuberosity, Sharpey' s fiber

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1.Background and purpose

Osgood-Sculatter-disease (OSD) is occurred at the pain and inflammation in the tibial tuberosity with intense excercise[1]. Likewise, the patella tendon for part of the quadriceps femoris muscle is attached at a tibial tuberosity. Furthermore, a patella tendon is embedded in the tibial tuberosity as the Sharpey's fiber[2] . From this, it was considered OSD is occurs that a tibial tuberosity is repeatedly pulled a tibial tuberosity to the quadriceps femoris muscle by sports activity.

On the other hand, the surface layer of tibial tuberosity was existed as fibrocartilage of a patella tendon. From this result, it is speculated that the surface layer of a tibial tuberosity has a structure that is resistant to traction [3]. Moreover, it is known that the Sharpey's fiber in a bone was change at the area and depth by physical activity [4,5] . Furthermore, the surface of bone was existed the osteoblast, a tendon and ligament fiber was secreted from fibroblast, and it is occurring the structural changes [6] .

However, increasing of physical activity with growing and structure of tibial tuberosity was not combined at the report,respectively. From these, in this study was going to focus on patella tendon attaching region of tibial tuberosity, tibial tuberosity of rat was histologically examining aimed at change in growing on structure.

2.Material and Method

2-1. Animals

The tibias of 3, 7 and 13-week-old rats (wistar strain, male) were excised after sacrificing by CO_2 gas. The proximal 1/3 part of those specimens was cut in sagittal direction and they were used for histological analyses. Furthermore, non-cutting specimens were used for preparations of a transparent specimen and a macroscopic specimen.

2-2. Making of microscopically observation specimens

The part of non-cutting specimens were fixed at 4% paraform aldehyde(PFA) and were used for observation of shape of the tibial tuberosity. The transparent specimen was made, using the other specimens by immersing into an alizarin-red and an alcian-blue staining fluid after fixation and dehydration. The shape of tibia was observed using the specimen treated by a sodium hypochlorite.

2-3. Non-decalcification and ground specimen embedded in resin

The specimens fixed were embedded in resin after dehydration and clearance. They

were cut in sagittal direction, were stained by toluidine-blue dye and were observed by a lightmicroscope. Thereafter, non-decalcification rosin sanding specimen was immersed in ethanol and acetone, it was gradually increased the concentration of rigolac rosin, embedded. After the polymerization is completed, sagittal specimen was observed by microscope for toluidine blue staining.

2-4. Scanning electron microscope specimen

Specimen of scanning electron microscope (SEM) was fixed by karnovsky fixing solution that contain in PFA and 5% glutaraldehyde. After that, it was immersed in 0.1M sodium phosphate buffer (pH7.4) 1% osmium solution. In the next, it was dehydrated and dried frees-dryer. Furthermore, this specimen was observed the tibial tuberosity by SEM that deposited in a vacuum with carbon and platinum.

3. Result

3-1. Microscopically observation specimens

A patella tendon was embedded in proximal of a tibial tuberosity. The fibers of patella tendon were confirmed to the periosteum via tibial tuberosity. (Fig.1a) In addition, when sagittal and horizontal sections area were observed, surface layer was white, deep layer was milky white. (Fig.1b) When a surface of tibial tuberosity was dried, the deep layer was sunk. But, surface layer was not sunk.

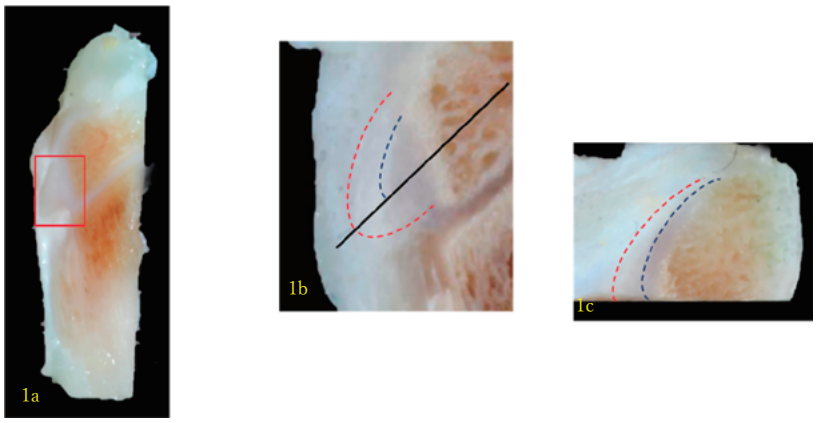

Fig.1 Microscopically observation in proximal of tibia. (Fig.1a) Fig.1b was magnified in red frame of Fig.1a. The cancellous bone side of Blue line was observed as deep layer of tibial tuberosity. Between a blue line and red line in surface layer. Fig.1c was grounded on black line of fig. I B, and tibial tuberosity was observed from above. As well as Fig.1b, tibial tuberosity was classified as surface and deep layer.

3-2. Sodium hypochlorite of microscopically observation specimens

A tibial tuberosity of 3-week-old rats were disappeared a tibia epiphysis by a sodium hypochlorite. (Fig.2a) A tibial tuberosity of 7-week-old rats were extended distally compared to 3-week-old rats. Furthermore, space of growth plate was slightly remained. (Fig.2b) In addition, tibial tuberosity of 13-week-old rats were extended distally compared to 7week-old rats, but growth plate was not almost existed. (Fig.2c) A tibial tuberosity of 13-week-old rats were larger than the 7week-old rats. (Fig.3)

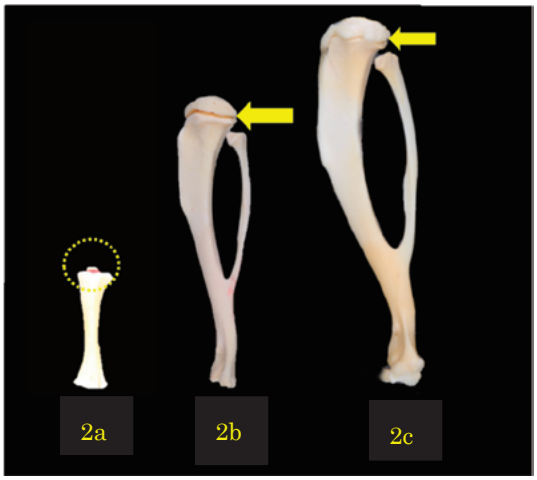

Fig.2 The Fig.2a, b and c were showed of tibial tuberosity of 3-7-13-week-old rats, respectively. A yellow dot circle was disappeared at the proximal tibia apophysis by sodium hypochlorite. The yellow arrows were showed on the growth plate.

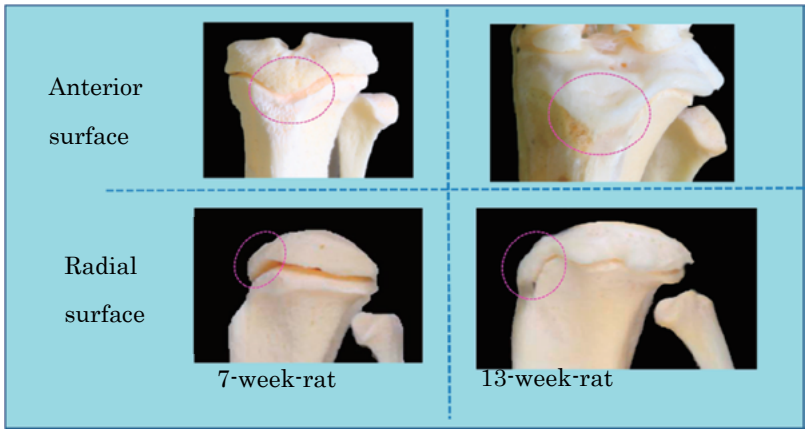

Fig.3 In the purple circles were showed of 7 and 13-week-old rats of tibial tuberosity, respectively.

3-3. Transparent specimen

A tibial tuberosity of 3-week-old rats were blue-dyed a whole it by alcian-blue staining fluids. (Fig.4a) A tibial tuberosity of 7-week-old rats were blue and red-dyed in surface layer of a tibial tuberosity. (Fig.4b) A tibial tuberosity of 13-week-old rats were less blue-dyed than the 7-week-old rats, and whole layer was red-dyed. (Fig.4c)

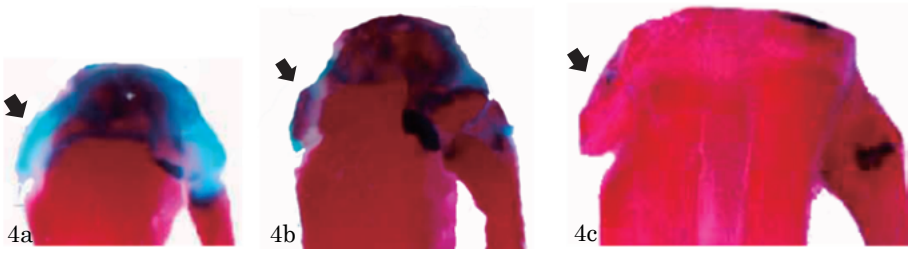

Fig.4 The Fig.4a, b and c were showed of tibial tuberosity of 3-7-13-week-old rats, respectively. The black marrows were showed at the tibial tuberosity. The Fig.4a,b and c were stained blue, red-blue, red at wholly there, respectively.

3-4. Non-calcification and ground specimen embedded in resin

The whole of a tibial tuberosity of 3-week-old rats are constructed in cartilage, and a patella tendon was slightly calcified that the surface layer of tibial tuberosity was sparsely embedded. (Fig.5a) A tibial tuberosity of 7-week-old rats were deep dyed in surface and superior, a patella tendon embedding in surface layer was constructed of fibrocartilage-like. (Fig.5b) Furthermore, deep layer of tibial tuberosity was identically dyed that growth plate and articular cartilage were constructed as the hyaline cartilage-like. The 13-week-old rats, the whole size of tibial tuberosity was swelled, it was calcified. (Fig.5c)

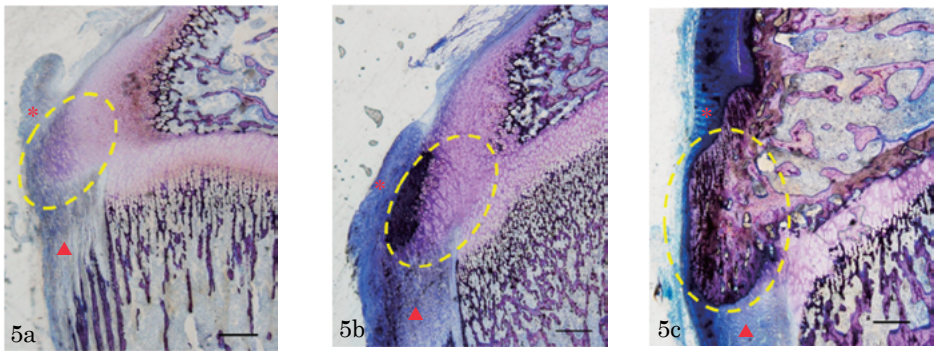

Fig.5 In the yellow dot circles were showed a tibial tuberosity (bar=200μm). The asterisks and trigones were recognized the patella tendon and the periosteum, respectively. The Fig.5a, b and c were indicated at the 3-7-13-week-old rats, respectively.

3-5. Scanning electron microscope specimen

When the surface layer of tibial tuberosity was magnified, non-calcification region was confirmed. (Fig.6b) Moreover, the cartilage lacunas of region were reticular arranged as fiber bundle. In addition, the cartilage lacunas were form a line for chondrocyte. (Fig.6c)

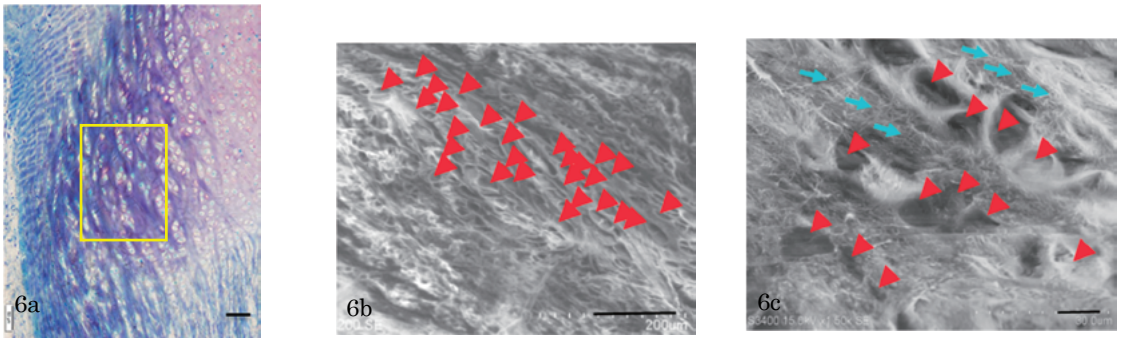

Fig.6a was showed of the tibial tuberosity (bar=50). Fig.6b was magnified in yellow frame of Fig.6a (bar=100). Fig.6c was magnified in yellow frame of VI.B (bar=100). The Red arrow heads and light blue arrows were recognized the cartilage lacunas and reticular fiber bundles.

4. Discussion

The transparent specimens were dyed by alizarin-red and alcian-blue staining fluids, respectively. This staining was red dyed a bone, and cartilage was blue dyed. The tibial tuberosity of 3,13-week-old rats were dyed the whole it for blue and red, respectively. On the other hand, a tibial tuberosity of 7-week-old rat was mixed the red and blue staining. The deep layer of a transparent specimens of the 3,7-week-old-rats were uncalcified state, and this region was same color the growth plate. From this result, the region of milky white of Fig.1b is thought that the non-calcification state was suit. The thickness of tibial tuberosity was increasing looked like with growing, but the part of calcified and non-calcified state is thought that at the near to merged states [7-9].

On the other hand, it is reported that skeletal tissues are developed with increase of mechanical stress [10]. From this study, it is considered that the cartilage was calcified by increase of mechanical stimulation with growing. Furthermore, it is known that the tibial tuberosity was direct pulled to the fiber of the patella tendon on the contraction of the quadriceps femoris muscles. In addition, by the study of Benjamin, it is reported that the joint movement was do exercise with the contraction at the skeletal muscle [11]. Moreover, metabolism of chondrocyte was affected by mechanical stimulation.[12]. From these reports, it is considered that the whole of tibial tuberosity was large and developed by the contraction

force of quadriceps femoris muscle, increase of mechanical stimulation and the increase of weight with growing of skeletal.

A tibial tuberosity in this study was matured that it was grown to distal direction with growing. Furthermore, the growth plate in posterior of tibial tuberosity was narrowed the site of growth plate with the skeletal development. This result was correspond that the characteristic of a tibial tuberosity is the tongue-like epiphysis[1]. The growth plate was classified as resting, proliferating, prehypertrophic, hypertrophic and terminal hypertrophic, respectively, it is affected long axis growing of bone[13]. From this, it is considered that tibial tuberosity was large to distal direction with growing of a bone with the development, and calcified.

Moreover, the surface and deep layers of tibial tuberosity were observed as white and milky white, respectively. In this results, sagittal and horizontal was identically resulted. The non-decalcified sanding specimen was observed and developed the epiphysis cancellous bone with the growing of skeletal, and the calcifying a tibial tuberosity. In previous research, it is reported that the hypertrophic layer region of chondrocyte was calcified. [14]. From these results, the tibial tuberosity was calcified from the deep layer with growing, as well as the apophyseal cancellous bone.

The tibial was separated after drying as milky white and white. In addition, the surface layer at densely embedding of patella tendon fiber and the deep layer at identically to dyeability of the growth plate were observed. On the other hand, the proteoglycan was existed into the cartilage matrix, it has including the water[15]. Furthermore, the growth plate is occurred as metachromasia by toluidine-blue dyeing[16]. From these, a tibial tuberosity is classified by the surface layer of slight the cartilage matrix and the deep layer of abundant the cartilage matrix. In addition, it is considered that the deep layer of tibial tuberosity was structured as hyaline cartilage-like, the surface layer was structured as fibrocartilage-like.

The tibial tuberosity of 3-week-old rat was whole constructed by cartilage, the Sharpey's fiber of surface layer was not mostly observed. But, this the region of 13-week-old rat was widely observed. Generally, the region to embedded into a tendon was known to "enthesis" [(2)], this region is existed as the fibrocartilage [(14)]. Furthermore, it is known that Sharpey's fiber was rigidly embedded into a bone. This result, the surface layer of tibial tuberosity was existed the fibrocartilage, this region was cannot pull out for calcifying.

The result of SEM was covered the cartilage lacuna in slim fibers. Moreover, the surface of collagen is existed the cartilage matrix. On the other hand, the surface of collagen was

existed as cartilage matrix[15-17] . Among them, proteoglycan was had as characterize of water holding property and viscoelasticity[18] . However, the surface of collagen masking by cartilage matrix was cleared by the sodium hypochlorite, it is thought the collagen fibers was disclosed.

5.Conclusion

It was understood that the tibial tuberosity was classified as surface and deep layer, it was whole calcified with development.

6.Committee of Animal Experiment and Ethics

This study was approved by Committee of Animal Experiment and Ethics for the research, Graduate School of Graduate School of Human Life Design, Toyo Univercity.

7.Acknowledgements

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発育期ラット脛骨における膝蓋靱帯付着部位の構造変化

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要旨

【背景】脛骨粗面は発育に伴う自重や活動性の増加に伴って筋からの牽引力が増大するが、それを受け止める腱付着部がどのような構造変化によって抵抗性を高めるかについては明らかにされていない。

【目的】これらのことから、本研究では脛骨粗面の膝蓋靱帯付着部に着目し、ラットのその部位における　構造上の発育変化を組織学的に検討することを目的とする。　方法：離乳期（３週齢)、発育期（７週齢)、成人後期（１３週齢）のwistar系雄性ラットを用い、安楽死させた後、脛骨を摘出し、種々の標本を作製した。

【結果】脛骨を乾燥させると、脛骨粗面は乳白色である深層と、白色である浅層が観察された。また、乳白色の部分は白色の部分に比べてへこんでいた。３週齢は脛骨粗面全体が軟骨組織で構成されていた。７週齢は３週齢と比べて、脛骨粗面が大きくシャーピー線維が多く埋入されていた。13週齢では、脛骨粗面全体が石灰化していた。

【考察】脛骨粗面を乾燥させると、深層部分が浅層に比べへこんでいた。一方、硝子軟骨にはプロテオグリカンが多く含まれ、これは、水を多く貯留し、軟骨に弾力を与える役割を持っている。脛骨粗面を乾燥させたことによって、貯留されていた水分が蒸発し、深層がへこんだと考えられる。このことから、脛骨粗面は硝子軟骨様である深層と繊維軟骨様である浅層の２つに大別でき、浅層と深層で異なる役割があると考えられる。加えて、発育に伴い脛骨粗面は大きさを増し、全体を石灰化していた。これは、発育に伴う自重や活動性の増加に

よって、膝蓋靭帯の線維が脛骨粗面から抜けないよう、抵抗性を高めたと考えられる。

【結論】脛骨粗面は浅層と深層に分類され、発育に伴い脛骨粗面全体が石灰化することが理解された。

キーワード：膝蓋靭帯、脛骨粗面、シャーピー線維